

1. A method for multiplexed analysis of a plurality of target nucleic acid sequences in a sample comprising the steps of:

providing, for each target nucleic acid sequence to be analyzed, at least one probe/primer molecule which probe/primer molecule includes a region of sequence substantially complementary to a sequence in the target nucleic acid sequence and a region that is not located at either terminus of the probe/primer and which includes a capture tag sequence;

forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule specific for a target sequence and that target sequence are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe specific for the target sequence, is generated, thereby producing a derivative nucleic acid suitable for evaluation;

evaluating the presence of one or more capture sequence tags.

2. The method of claim 1, wherein the derivative nucleic acid molecules are analyzed by hybridizing the tag sequences to capture probes which are spatially separated.

3. The method of claim 2, wherein the capture probes are partially duplex probes with capture tag-complementary single stranded overhangs

4. The method of claim 2, wherein the capture tags are disposed on beads.

5. The method of claim 2, wherein the capture tags are disposed on an ordered array.

6. The method of claim 2, wherein the derivative nucleic acid is ligated to a capture probe and then washed.

7. A method of providing a derivative nucleic acid having single-strand overhang

suitable for analysis comprising:

providing a first and second primer,

wherein said first primer includes, in the order of 5' to 3',

a first region which includes a universal primer sequence,

a second region which includes a capture tag sequence and a
cleavage site, and

a third region which can which can hybridize to a first region on
the target nucleic acid sequence,

wherein said second primer includes, in the order of 5' to 3',

a first region which includes a universal primer sequence,

a second region which includes a capture tag sequence (the capture
tag sequence on the second primer can be the same or different from that of
the capture tag of the first primer) and a cleavage site, and

a third region which can which can hybridize to a second region on the
target nucleic acid sequence,

forming a reaction mixture which includes the first and second primers and the
target nucleic acid, and using the target as a template, extending the primers along the
target nucleic acid, to produce an extended target strand, which includes, in order, a
universal primer sequence, a capture tag sequence, target sequence, a capture tag
sequence, and a universal primer sequence;

contacting an extended target strand with a universal primer which binds to the
universal primer sequence and extending the universal primers along the extended target
strand to synthesize a extended target strand which includes, in order, a universal primer
sequence, a capture tag sequence, target sequence, a capture tag sequence, and a universal
primer sequence;

cleaving at the cleavage site of one or both ends of a double stranded extended
target molecule to provide a derivative nucleic acid which includes a double stranded

molecule having an overhang which includes the capture tag sequence, preferably at one or both of the 3' and 5' termini

thereby producing a target molecule having an overhang.

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8. The method of claim 7, wherein the method further includes multiplexed reactions and the reaction mix includes a second target, and a third and a fourth primer are included in the reaction mix,

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wherein the third primer includes, in the order of 5' to 3',

a first region which includes a universal primer sequence,

a second region which includes a capture tag sequence (which is different from the capture tag sequence on one or both of the first and second primers) and a cleavage site, e.g., a cleavage site for cleavage by a restriction enzyme, and

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a third region which can hybridize to a first region on the second target,

wherein the fourth primer includes, in the order of 5' to 3',

a first region which includes a universal primer sequence;

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a second region which includes a capture tag sequence and a cleavage site, e.g., a site for cleavage by a restriction enzyme, and

a third region which can which can hybridize to a second region on the second target,

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forming a reaction mixture which includes the first, second, third and fourth primers and the two targets, and using the second target as a template, extending the third and fourth primers along the second target, to produce an extended second target strand, which includes, in order, a universal primer sequence, a capture tag sequence, target sequence, a capture tag sequence, and a universal primer sequence (the extended second target will include a capture tag sequence which is different from that on the extended first target);

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contacting an extended second target strand with a universal primer which bind to the universal primer sequence and extending the universal primers along the extended second target strand to synthesize a extended second target strand which includes, in order, a universal primer sequence, a capture tag sequence, target sequence, a capture tag sequence, and a universal primer sequence (wherein at least one capture tag is different from a capture tag on the first extended target);

cleaving at the cleavage site of one or both ends of a double stranded second extended target molecule to provide a derivative nucleic acid which is a double stranded molecule having an overhang which include a capture tag sequence, at one or both of the 3' and 5' terminus.

9. The method of claim 7, wherein the derivative nucleic acid molecules are analyzed by hybridizing the tag sequences to capture probes which are spatially separated.

10. The method of claim 9, wherein the capture probes are partially duplex probes with capture tag-complementary single stranded overhangs.

11. The method of claim 9, wherein the capture tags are disposed on beads.

12. The method of claim 9, wherein the capture tags are disposed on an ordered array.

13. The method of claim 9, wherein the derivative nucleic acid is ligated to a capture probe and then washed.

14. A method of using ligatable probes to provide a nucleic acid having single-strand overhangs suitable for analysis comprising:

providing a first and second probe,

wherein said first probe includes, in the order of 5' to 3',

a first region which includes a universal primer sequence;

a second region which includes a capture tag sequence and a cleavage site, and

a third region which can which can hybridize to a first region on the target nucleic acid,

5 wherein said second probe/primer includes, in the order of 3' to 5',

a first region which can which can hybridize to a second region on the target nucleic acid,

10 a second region which includes a capture tag sequence (the capture tag sequence on the second probe/primer can be the same or different, from that of the capture tag of the first probe/primer) and a cleavage site, and

a third region which includes a universal primer sequence;

15 forming a reaction mixture which includes the first and second primers and the target nucleic, under conditions wherein the first and second probes are joined if the target is of a first sequence and not joined if the target is of a second sequence, to produce a joined probe, which includes, in order, a universal primer sequence, a capture tag sequence, target sequence, a capture tag sequence, and a universal primer sequence, preferably in the order 5' to 3';

20 cleaving at the cleavage site of one or both ends of a double stranded extended target molecule to provide a derivative nucleic acid which is a double stranded molecule having overhangs which include the capture tag sequence at one or both of the 3' and 5' termini,

thereby producing a target molecule having overhangs.

25 15. The method of claim 14, wherein the method further includes multiplexed reactions and the reaction mix includes a second target, and a third and a forth probes are included in the reaction mix,

wherein said third probe includes, in the order of 5' to 3',

a first region which includes a universal primer sequence;

a second region which includes a capture tag sequence (which is different from the capture tag sequence on one or both of the first and second probe) and a cleavage site, and

a third region which can which can hybridize to a first region on the target nucleic acid,

5 wherein said fourth probe includes, in the order of 5' to 3',

a first region which can which can hybridize to a second region on the target nucleic acid,

a second region which includes a capture tag sequence and a cleavage site, and

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a third region which includes a universal primer sequence;

forming a reaction mixture which includes the first, second, third and fourth probe and the two targets under conditions wherein the third and fourth probe are joined if the second target is of a first sequence and not joined if the second target is of a second sequence, to produce a second joined probe, which preferably includes, in order, a universal primer sequence, a capture tag sequence, target sequence, a capture tag sequence, and a universal primer sequence, preferably in the order 5' to 3';

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cleaving at the cleavage site of one or both ends of a double strand joined probe to provide a derivative nucleic acid which is a double stranded molecule having overhangs which include the capture tag sequence at one or both of the 3' and 5' termini.

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16. The method of claim 14, wherein the derivative nucleic acid molecules are analyzed by hybridizing the tag sequences to capture probes which are spatially separated.

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17. The method of claim 16, wherein the capture probes are partially duplex probes with capture tag-complementary single stranded overhangs.

18. The method of claim 16, wherein the capture tags are disposed on beads.

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19. The method of claim 16, wherein the capture tags are disposed on an ordered

array.

20. The method of claim 16, wherein the derivative nucleic acid is ligated to a capture probe and then washed.

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21. A method of providing a nucleic acid with single strand overhang suitable for multiplex analysis comprising:

(1) providing a sample which includes one or a plurality of target nucleic acid sequences;

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(2) providing a first single stranded linear probe, wherein the first single-stranded linear probe includes,

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at one terminus, a first region which is complementary to a first region on a first target, and at its other terminus a second region which is complementary to a second region on a first target, wherein the first and second region on the first target can be directly or can be separated by one or more nucleotides (upon hybridization of the first and second regions to the target, the termini of the probe can be joined, thus the probe will be circularized only when the target sequence for which the probe is specific is present in the sample),

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a cleavage site

a capture tag sequence, wherein the cleavage site and capture tag sequence are disposed such that cleavage results in a single-stranded overhang, at one or both of the 3' and 5' termini, and

a universal primer sequence;

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(3) providing a second single stranded linear probe, wherein the second single-stranded probe includes,

at one terminus, a first region which is complementary to a first region on a second target, and at its other terminus a second region which is complementary to a second region on a second target, wherein the first and second region on the first target can be directly or can be separated by one or more nucleotides,

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a cleavage site

a capture tag sequence, which capture sequence can differ in sequence from the capture tag on the first single stranded probe, and wherein the cleavage site and capture tag

sequence are disposed such that cleavage results in a single-stranded overhang, at one or both of its 3' and 5' termini, and

a universal primer sequence, which is preferably, of the same sequence as the universal primer sequence on the first single stranded probe;

5 contacting the first single stranded probe with the first target and the second single stranded probe with the second target under conditions which allow the circularization of the a single stranded circular probe if to be circularized if a target is present which is homologous to its terminal target binding regions;

10 contacting the first and second probes with a universal primer under conditions which allow rolling circle amplification and produce double stranded amplification product;

 cleaving the double stranded amplification product at cleavage sites, e.g., with a restriction enzyme to provide cleaved product having a capture tag sequence at the terminus of a single stranded overhang.

15 22. The method of claim 21, wherein the derivative nucleic acid molecules are analyzed by hybridizing the tag sequences to capture probes which are spatially separated.

20 23. The method of claim 22, wherein the capture probes are partially duplex probes with capture tag-complementary single stranded overhangs.

24. The method of claim 22, wherein the capture tags are disposed on beads.

25 25. The method of claim 22, wherein the capture tags are disposed on an ordered array.

26. The method of claim 22, wherein the derivative nucleic acid is ligated to a capture probe and then washed.

30 27. A method of providing a nucleic acid with single strand overhang suitable for detecting one or more genetic events in a sample comprising:

(a) providing a target nucleic acid having a first and second region, wherein the two regions preferably overlap;

providing an invader probe which is complementary to the first region of the target,

5 providing a signal probe having, in the 5' to 3' direction, signal sequence, a capture tag sequence, and a region complementary to the second region of the target nucleic acid;

(b) contacting the target sequence with the invader probe and the signal probe, under conditions wherein the invader probe and an end, e.g., the 3' end of the signal probe are annealed to the target nucleic acid sequence so as to create a cleavage structure having a single-stranded arm which includes the capture tag;

10 (c) cleaving the first cleavage structure under conditions such that cleavage of the cleavage structure occurs at a site located within the signal probe in a manner dependent upon the annealing of the invader and signal probes on the target nucleic acid such that cleavage liberates the single-stranded arm of the cleavage structure to generate a derivative nucleic acid which has the capture tag sequence at a terminus;

15 thereby providing a derivative nucleic acid suitable for analysis.

28. The method of claim 27, wherein the method further includes multiplexed reactions and the reaction mix includes a second target, and a third and a forth probes are included in the reaction mix, wherein,

20 the second invader probe is complementary to a first region of a second target, the second signal probe includes, in the 5' to 3' direction, signal sequence, a capture tag sequence (which is different in sequence from the capture tag sequence on the first signal probe), and a region complementary to a second region of the second target nucleic acid;

25 (b) contacting the second target sequence with the second invader probe and the second signal probe, under conditions wherein the second invader probe and an end, e.g., the 3' end of the second signal probe are annealed to the second target nucleic acid sequence so as to create a second cleavage structure having a single-stranded arm which includes the capture tag;

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(c) cleaving the second cleavage structure under conditions such that cleavage of the second cleavage structure occurs at a site located within the second signal probe in a manner dependent upon the annealing of the second invader and signal probes on the target nucleic acid such that cleavage liberates the single-stranded arm of the cleavage structure to generate a second derivative nucleic acid which has the capture tag sequence at a terminus;

29. The method of claim 27, wherein the derivative nucleic acid molecules are analyzed by hybridizing the tag sequences to capture probes which are spatially separated.

30. The method of claim 29, wherein the capture probes are partially duplex probes with capture tag-complementary single stranded overhangs.

31. The method of claim 29, wherein the capture tags are disposed on beads.

32. The method of claim 29, wherein the capture tags are disposed on an ordered array.

33. The method of claim 29, wherein the derivative nucleic acid is ligated to a capture probe and then washed.

34. A plurality of primers as described in claim 1, 7, 14, 21, or 27.

35. A plurality of derivative nucleic acids as described in claim 1, 7, 14, 21, or 27.

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